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PROTECTION OF CAMELS FROM BRUCELLOSIS

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The susceptibility of camels to brucellosis infection was first reported
in the USSR by Stepanov (1931) and Lamakin (1932).

Abusov experimentally detected brucella of the type abortus bovis in
camels, and determined for the first time the normal and pathological titers
of camel serum. Ostrovidov stressed the high sensitivity of camels to allergen.
However, the many problems of brucellosis infection in camels and the problems
of seroallergic diagnosis are still insufficiently studied and explained.
It is necessary to develop those methods of diagnosis which would be effective
and practical for extensive use.

In a test group of camels, brucellosis was detected serologically in
15 percent of the cases (2 percent of the pregnant camels aborted).

Study of the Normal and Pathological Agglutination Titer of the Blood
of Camels with the Brucellosis Antigen

The reaction of agglutination was studied by Zhalobovskiy and Pal'gov
on blood serum of various groups of camels by a uniform system using the
standard antigen.

The reaction was set up in one-milliliter volumes in serum dilutions
of 1:10:20:40:80:160.

The results of a double-checked agglutination reaction showed that the
highest percent of positive reactions was produced by the 1:10 dilution for
the serum of camels of all groups except the brucellosis group.

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As the degree of dilution is increased, the number of positive reactions is sharply decreased in all groups, independently of the sex or age (with the exception of the brucellosis group) until negative results are produced in the 1:40 dilution. It is quite the contrary in the brucellosis group, since the 1:40 dilution produces a great number of positive reactions, and in the 1:80 dilution a maximum of 35 percent is reached. A relatively high percent of positive reactions is found on further dilution until the terminal titer is reached; in the 1:200 dilution, 20 percent; the 1:400, 13 percent; and the 1:800, 8 percent.

In subsequent dilutions the agglutination phenomena was not found. The highest percent of positive reactions (three and four checks) was observed in the 1:80:160 dilutions. In the lower dilutions, 1:10:20, the percent of serum producing the index is one-third of that explained phenomenon of the proagglutinoid zone.

The Importance of Agglutination Reaction in the Diagnosis of Brucellosis in Camels

In order to study this problem the same authors made an examination of a test group of camels with brucellosis.

In the first examination the positive and doubtful reactions totaled 38 percent, and in the second, 43 percent. Concurring results of the two examinations were received in 77 percent of the cases.

Of the number of camels which had shown positive agglutination reactions, 70 percent reacted positively in the second examination. Of those camels which had had negative reactions, 87 percent reacted negatively in the second examination. From among those camels which had had doubtful agglutination reactions, 82 percent reacted positively and 18 percent negatively in the second examination.

This data shows that the agglutination reaction for brucellosis in camels is specific but that it frequently drops out. A pronounced agglutination reaction evaluated by the four-way check method occurs more rarely than by the double or three-way check and it is shown mostly in the 1:50:100 dilutions.

The Complement Fixation Reaction as a Method for Diagnosis of Brucellosis in Camels

The reaction was studied by Pal'gov and Zhalobovskiy with serum in four doses: 0.1-0.05-0.025-0.01 milliliter with the RKKA brucellosis antigen of the Scientific Research Veterinary Institute, with a control for nonspecific inhibition. All the serums were studied by both the agglutination reaction and the complement fixation reaction for mutual checking.

In the brucellosis group it was noted that the negative reactions for the 0.01 milliliter dose were three times more numerous than for the 0.1 milliliter dose, but that the positive reactions on the contrary were $1\frac{1}{2}$ times less. Healthy camels did not exhibit nonspecific inhibition of hemolysis even in doses of 0.2-0.4 milliliter.

The reaction with all doses of serum proceeded clearly in the order of dilution. It was established that all serums in doses of 0.025-0.01 milliliter with the inhibition of hemolysis in 2.5 (three- and four-way check lower dilutions, that is, 0.1-0.05 milliliter doses) constantly maintain their positive character.

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The noted regularity of the courses of the complement-fixation reaction and the absence of inhibition of hemolysis in the lower dilutions of serum showed the unsuitability of the complement-fixation reaction in doses of 0.025-0.01 milliliter. For diagnostic titer of the serums, doses of 0.1-0.05 milliliter should be considered as producing inhibition of hemolysis for negative control.

Comparison of parallel results of the complement-fixation reaction and the agglutination reaction showed the superiority of the former.

The stability of the index of the two serum reactions is not at all similar. Two studies of the serum with a month's interval between showed that the index of the complement-fixation reaction was almost unchanged. Concurring results for the complement-fixation reaction were received in 98.9 percent of the cases, and for the agglutination reaction in 77.5 percent of the cases, while the complement-fixation reaction was apparent in $2\frac{1}{2}$ times more camels with brucellosis than the agglutination reaction.

Allergic Methods of Diagnosis

Allergens were injected intracutaneously in the region of the dewlap near the shoulder joint. On the basis of thousands of measurements, Pal'gov had established that the thickness of the fold of skin at this point in camels at the age of 3 years or older was, on the average, 0.75 centimeter, and varied from 0.6 to 0.9 centimeter.

Brucellizate was used for the first two groups of camels and abortin for the second along with simultaneous investigation with the agglutination reaction and the complement-fixation reaction, with the following results (percent):

<u>Reaction to</u>	<u>Young Camels</u>	<u>Adult Camels</u>
Brucellizate	64.6	81.1
Abortin	60.2	83.5
Complement-fixation reaction	64.8	89.4
Agglutination reaction	38.7	52.1

In the number of positive results the test with allergens in the adults is only slightly exceeded by the complement-fixation reactions and significantly excels the agglutination reaction.

The test with allergens in the young camels as opposed to that in the adults showed a sharp reduction of 25 percent in number of positive reactions in the case of the young camels.

Brucellizate produced a relatively higher percent of reactions than abortin, nearly 5 percent.

Control camels of an uninfected group produced negative results for brucellosis using brucellizate, the complement-fixation reaction, and the agglutination reaction, at the same time that the test with abortin produced 21.8 percent positive and doubtful reactions.

If we are to speak of the specificity of allergen, then, based on indications of the control group, we should state a preference for brucellizate.

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Methods of Sanation

Because of improved accuracy of the diagnostic titer of serums in the agglutination reaction for the detection of brucellosis infections in camels, the specificity of the indexes of the complement-fixation reaction, and the demonstrated high sensitivity of camels with brucellosis to the intracutaneous injection of allergens, it is possible to establish that the seroallergic complex agglutination reaction -- complement-fixation reaction, agglutination reaction -- brucellizate is an accurate method for the diagnosis of brucellosis in camels, since they supplement each other. This same complex can serve as a control for healthy and recovered camel stocks.

Timely detection of animals with brucellosis by the serocomplereactions permits their isolation from healthy animals and the arrangement of measures for rearing healthy young camels which were born of camels with brucellosis.

Abortus of brucellosis origin were observed in the first year among the group of camels with brucellosis which was studied over the courses of 5 years. It was shown that aborts as a rule developed in the female camels in the first half of pregnancy. Bacteriological study of the aborted fetuses showed that the strains cultivated by them were identical to strains of brucella abortus bovis in their morphological, cultural, and antigenic features.

Subsequent investigations showed that 3-4 month quarantine and two-way check of the agglutination reactions of the camels does not insure detection of all the animals with brucellosis, but that only the introduction of auxiliary investigation by the complement-fixation reaction increases the effectiveness of the detection.

It was also shown that the concurrence of positive results by both reactions was observed in 32 percent of the cases tested, negative agglutination reactions with positive complement-fixation reactions in 57 percent, and positive agglutination reactions with negative complement-fixation reactions in 11 percent.

A study of the abort cases showed that among animals which were in the group for more than 3-4 years, the quantity of aborts is reduced, and that at that time repeated aborts were only 20 percent of those among camels with fresh infections.

However, the percent reacting positively in the brucellosis group for 5 years remained nearly constant: by complement-fixation reaction not less than 75 percent by agglutination reaction 10-50 percent.

The clinical form of brucellosis was rarely encountered in the camels.

Characteristics by year: In the first year of the investigation, the epizootological situations was the most intense. Ten aborts of brucellosis origin were observed.

In the second year, 63 percent of the camels reacting positively in the first year still did. There were nine aborts of brucellosis origin.

In the third year, 47 percent reacted positively and there were three aborts of brucellosis origin.

In the fourth year, only 3.7 percent of the camels reacted positively and no aborts due to brucellosis were recorded.

At the end of the fifth year, there were signs of recovery and the group was considered conditionally safe.

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The following year, due to negative results of the reactions, the absence over the year's period of abort cases, retention of afterbirths, or premature births of brucellosis origin, or any of other clinical indications of brucellosis, the conditionally safe group was declared free of brucellosis.

Epizootological Importance of Age Groups

The possibility of producing healthy young from animals with brucellosis (large horned cattle, and sheep) has been demonstrated in special experiments and confirmed in wide practice. Analogous work in the case of young camels has not yet been conducted. Besides that, a series of questions on the epizootological importance of age groups of camels and the course of their immunobiological reactions is still unexplained.

Instructions on the separation of young camels from their mothers in the third to sixth month, based entirely on analogy with large horned cattle, do not have any experimental foundation.

Camel raisers confirm that separation of young camels from their mothers earlier than the seventh or eighth month has an unfavorable influence on their development. Feeding by concentrates cannot compensate for the nutrient substances the young camel gets from its mother's milk.

In our tests, the separation of young camels both from healthy mothers and from those with brucellosis is usually done in the ninth to tenth month.

The problem of freedom from brucellosis of the young animals born to camels with brucellosis is solved, as is known, after the advent of sexual maturity and breeding. This condition is of considerable importance in connection with subsequent investigations on the susceptibility of the young animals to brucellosis and the particular course of the infection in them which frequently appears only after sexual maturity.

The studies we conducted on this problem dealt with young camels born to mothers of seven different ages which had brucellosis. The methods of study were the same. The first test group was made up of young camels 1 to 3 years old.

This collected group showed in the first year of the test that it was not free of brucellosis. Twelve percent reacted positively for brucellosis. In the period of sexual maturity and the first pregnancy, the number reacting positively reached 13.3 percent and two abortions of brucellosis origin were noted.

The negative sides of the rearing of young camels in this group consisted in the combination of young of various ages, separating the young camels from their mothers at a late point (after their tenth month), and raising them with the brucellosis group.

We changed the system of rearing the young camels, using as a basis the isolated maintenance of them by age together with timely separation from their mothers.

During the period of our tests, we studied the course of the immunobiological reaction in the young progeny of brucellosis mothers from the day of their birth and the susceptibility to brucellosis infection at all ages of these young camels.

At the same time, we were interested in the ratio reacting positively to brucellosis in relation to their age.

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In order to study the dynamics of the immunobiological reactions of the young born to mothers with brucellosis, we examined them each month from the day of their birth until they were 10 months old (see Table 1).

After birth, the number reacting to the agglutination reaction is insignificant (6 percent, but on the other hand, the number reacting to the complement-fixation reaction is 82 percent. In the course of time, the percent reacting to the agglutination fluctuates between four and nine. However, the number of reactions to the complement-fixation reaction drops regularly from 82 percent shortly after birth to 8.5 percent in the tenth month.

Of the total number of young camels having positive reactions by the complement-fixation reaction at the time of their birth, the following give negative reactions in subsequent tests: at one month, 60 head; at 2 months, 22; at 3 months, 32; at 4 months, 2; at 5 months, 16; at 6 months, 17; and at 7 months, 44.

A cycle restoring them gradually to their initial condition was apparently run. Of the number which lost their complement-fixation reaction, 4 returned to the positive phase in the fifth month, 30 in the sixth, 44 in the seventh, 6 in the eight, 2 in the ninth, and 12 in the tenth.

Furthermore, a gradual increase in agglutinins and complement-fixation substances was noted when the young camels remained in the brucellosis herd beyond the seventh to eighth month.

The group of young camels born of healthy mothers and studied at the same time as those of mothers with brucellosis served as a control. The test showed that the young camels born of healthy mothers for the course of the whole year invariably had negative agglutination and complement-fixation reactions. These contrasting indications were decidedly a point in favor of using the seroallergic complex of the two reactions.

For a comparative examination of the course of the immunobiological reactions by age characteristics, we present data of an investigation of camels with brucellosis (see Table 2). It is obvious that this data differs radically from that on young camels in Table 1. The percent reacting by the agglutination reaction in one-month-old camels was insignificant (6 percent), and in adults, relatively high. The first indexes of the complement-fixation reaction nearly agreed, 82 percent in the young and 89 percent in the adults. However, the percent of adults reacting positively by the complement-fixation reaction remained constant for the whole year (90-85), but there was a gradual reduction in the young from 82 percent to 8.5 percent by the end of the year.

The percent of adults reacting to the agglutination reaction fell in the spring to 7 but rose again in the fall to 42. At the same time, the complement-fixation reaction index was stable regardless of the time of year. From the exposition it is apparent that there has been preliminarily established a reduction of the resistance of young camels to brucellosis in the seventh month. In order to confirm this condition and test the specificity of using the seroallergic complex, biological tests were conducted on young camels, 9 to 16 months of age, which had positive seroallergic reactions and which were born of camels with brucellosis.

Biological tests on guinea pigs gave positive results in 70 percent and experimental transmission in 50 percent of the cases. The majority of infected guinea pigs reacted positively on the 30th-40th and some on the 60th day, and those to which it was experimentally transmitted on the 20th-30th day.

In both cases a culture of the type *Brucella abortus bovis* was isolated.

The index of the complement-fixation reaction concurred with the brucellizate index in 90 percent of the cases.

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By the data of the seroallergic reactions and serum testing, we diagnosed brucellosis in experimental camels from their ninth month.

Summing up the results of our tests, we are convinced that for the production of healthy young animals it is necessary to isolate those with negative seroallergic reactions from mothers with brucellosis not later than their seventh or eighth month, and to rear them by isolated age group (by the year of their birth) and systematically study them until the age of sexual maturity and their first safe delivery.

Using this system of sanitary measures with the young, we bred healthy camel stock in 3 years.

The experimentally devised system which we propose of "conveyer" rearing by isolated age groups can be recommended for wide application in camel-raising farms.

Conclusions

1. The height of the normal agglutination titer of blood serum of camels does not exceed 1:20:25, and the pathological titer is 1:40:50 and higher.
2. Diagnostic doses for studying the complement-fixation reaction for brucellosis in camel serum are 0.1 - 0.05 milliliter.
3. The serological complex test conducted on camels showed that while complement-fixation reaction is superior to the agglutination reaction in sensitivity and consistency it is not able to replace it completely. Both reactions are specific for brucellosis and supplement each other.
4. Brucellizate has a specificity in carrying out allergy tests for brucellosis on camels, but is not quite as good as the complement-fixation reaction. Practical use of brucellizate in camel raising under herd conditions is very complicated and is not superior to serological methods for our purposes.
5. Complement-fixing substances produced by the bodies of camels with brucellosis are more stable than agglutinins and are observed earlier than agglutinins in blood sera.
6. Strains of brucella taken from aborted fetuses are identical to strains of Brucella abortus bovis in their morphological, cultural, and antigenic properties.
7. Aborts of brucellosis origin in camels as a rule are observed in the first half of pregnancy.
8. In camels the clinical symptoms of brucellosis are rarely observed. The disease more often occurs without symptoms.
9. The systematic use of the seroallergic complex for conducting the whole cycle of veterinary-sanitary measures achieves effective results in eliminating, in the sanitary sense, brucellosis from the farm.
10. The allergic property is less developed in camels under 3 years of age than in older ones.
11. Agglutinins and complement-fixation substances are found in young camels born of mothers with brucellosis from the day of their birth and disappear in the first 4 or 5 months.
12. Young camels born from their mother's first or second delivery as a rule react positively to the agglutination reaction with a high titer of 1:600:800.

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13. According to the data of the experiment, the resistance to brucellosis infection decreases in young camels at about the eighth month. At this time, they should be separated from mothers with brucellosis, reared in isolated age groups and subjected to investigation until sexual maturity and the first safe delivery.

Age (in mo)

Table 1

Reaction	After Birth	<u>1</u>	<u>2</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>
Agglutination (%)	6.0	--	--	4.3	9.0	7.5	8.6	7.0	6.0
Complement Fixation (%)	82.0	56.0	46.6	41.4	32.2	19.0	16.3	12.0	8.5

Month

Table 2

Reaction	<u>Aug</u>	<u>Sep</u>	<u>Apr</u>	<u>May</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>
Agglutination (%)	35	35	22	7	42	34	40
Complement Fixation (%)	89	89	90	85	90	80	83

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